INHIBITION OF LOCOMOTION IN PARAMECIUM AND OBSERVATIONS ON CERTAIN STRUCTURES AND INTERNAL ACTIVITIES.[1]

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The study of *Paramecium* under magnifications sufficiently high to permit of observations on the activities within the living organism has been considerably hindered in the past by the excessive motility which this protozoön exhibits. A number of methods, physical and chemical, have been employed in attempting to overcome this difficulty; yet all of these methods are open to the objection that they are transient, or that they hinder the observation of certain activities, or even that they are severely limited in their application by the sensitiveness of the organism to protoplasmic poisons and osmotic changes.

Of the physical methods for quieting paramecia the following may be mentioned: (1) entanglement in zoöglea or other debris; (2) retardation of locomotion in gelatinous media; (3) inactivation at low temperature. The objections to these methods are patent: Entanglement affords an occasional arrest of activity; but in the infrequent cases where it is by chance efficient the animal is often obscured from view. Retardation in such media as quince seed jelly or gelatin is effective only when the gel is sufficiently thick to prevent the entrance of food particles at the peristome, in which case observation on this activity is impossible. Chilling may greatly reduce locomotion, but internal movements are at the same time unavoidably retarded.

The chemical narcotizing agents in general use for the lower animals are also in certain respects unsatisfactory for *Paramecium:* Methyl alcohol and chloral in concentrations sufficiently high to give any noticeable effect render the animal abnormal in appearance before paralysis sets in. Ethyl alcohol is open to the same objection, though to a less degree. Chloretone in concentrations

[1] This paper was prepared under the direction of Professor S. O. Mast.

around one tenth per cent. is serviceable; however, the margin between the narcotizing and lethal doses is so small that great precautions are necessary in its use.

In studying the action of several of the aliphatic alcohols the writer has observed that in propyl and iso propyl alcohols of certain dilution a balance of effects obtains such that the external (locomotor) cilia of *Paramecium* are inactivated before the internal activities of the animal are changed. Locomotion, therefore, can be reduced or stopped without appreciably affecting any of the visible activities which obtain within the animal itself.

In narcotization a single paramecium is added to a drop of the alcohol solution; or, better, one volume of a rich culture of mixed races is quickly and intimately combined with one volume of the alcohol of double the desired strength. For class demonstrations a number of cubic centimeters should be prepared by the latter method half an hour in advance and distributed to the students for study. The narcotized protozoa may then be observed under a cover glass or directly with a water immersion lens. When the animals come in contact with the stronger alcohol a few are immediately killed, while the majority at first exhibit avoiding reactions—they move erratically, become frequently bent in at the peristome, and may discharge a few trichocysts; then, as these reactions to the new stimulus cease, they become paralyzed and settle to the bottom. This final effect obtains in approximately half an hour from the application of the alcohol, the time varying with the race and condition of the individuals. The completely paralyzed paramecia exhibit little or no ciliary activity on the body, yet the cilia in the oral groove and at the peristome maintain a vigorous and coördinated movement; the undulating membrane of the œsophagus continues to direct the ingestion of food in approximately the normal manner; and the activities of the contractile vacuoles, food vacuoles and streaming protoplasm remain apparently normal for an extended period. With careful manipulation the narcosis can be continued for four or five hours, and these activities can be *gradually* retarded by heavy or prolonged narcotization. Thus it is evident that the study of *Paramecium* is greatly facilitated by the use of propyl alcohol as a narcotizing agent.

Paralysis is induced by the action of the alcohol on the cilia.

These may be inactivated, incoördinated or cytolyzed. If the narcotized animals are removed to a normal medium, those of them which are not too severely affected slowly recover. Recovery is indicated by a beginning of locomotion accompanying the reappearance of the body cilia. Whether these are actually regenerated or merely reëxtended and stiffened for swimming has not been determined.

TABLE I.

SHOWING THE OPTIMUM CONCENTRATIONS OF PROPYL ALCOHOL REQUIRED TO INHIBIT LOCOMOTION IN EIGHT PURE LINES OF *Paramecium*.

The percentages by volume are estimated from the conversion equivalents for ethyl alcohol.

Number of Culture.	Normal Propyl Alcohol.		Iso Propyl Alcohol.	
	Per Cent. by Weight.	Per Cent. by Volume.	Per Cent. by Weight.	Per Cent. by Volume.
P. caudatum 1	1.0	1.3	2.0	2.5
2	1.5	1.9	2.5	3.1
3	2.0	2.5	3.0	3.8
4	2.0	2.5	3.0	3.8
5	2.5	3.1	3.0	3.8
P. aurelia 1	2.0	2.5	3.5	4.4
2	2.5	3.1	3.0	3.8
3	2.5	3.1	3.5	4.4
Average	2.0	2.5	2.9+	3.7

It appears that the permissible variation from the optimum narcotizing concentration is greater with iso and normal propyl alcohols than with any of the quieting agents heretofore employed. With different cultures, however, the narcotizing concentration varies considerably, as the results presented in Table I. indicate. This table gives the optimum concentrations of the two propyl alcohols when used on various typical pure line cultures of *Paramecium caudatum* and *Paramecium aurelia*. It should be noted that the former species narcotizes more satisfactorily than the latter, and that wild cultures, or mixed cultures of pure lines, are more satisfactory than single pure lines of either species, since among the varied specimens of the mixed "populations" a considerable number which will narcotize properly can be much more readily found than in the more uniform specimens of the pure stocks.

By appropriate concentrations of propyl alcohol other microscopic animals can be narcotized, though with less advantage than *Paramecium*. *Paramecium bursaria* is paralyzed, but cyclosis also is stopped. The erratic movements of *Oxytricha* continue, though less frequently and energetically. *Spirostomum* gives acute avoiding reactions, twisting and bending so sharply at the peristome that the body is frequently severed. The cilia on the fragments continue to beat as long as any ectoplasm remains, but those on unbroken animals that are narcotized quickly disappear. *Stentor* becomes quiet, and some of the cilia at either extremity continue to beat; it contracts so much, however, that little can be seen within. *Colpidium* and *Colpoda* become quiet, but the internal activities also cease. The contractile flagellates—*Euglena, Peranema* and *Distigma*—are stimulated to pronounced euglenoid contortions; their flagella are frequently lost, and may be grown again in fresh media. In the case of *Peranema* the writer has observed the flagellum actually regenerate about one fifth its length in three hours. Of the rotifers, *Monostyla* narcotizes satisfactorily; many of the others, however, merely undergo contortions up to the point of death. The various larvæ and nematodes that were observed gave likewise results of no special interest. In general, then, it may be said that while propyl alcohol gives a more or less effective narcotization in a number of microörganisms, *Paramecium* is the only form studied in which internal activities of a strikingly obvious character obtained after narcotization.

Some paramecia, narcotized in accordance with the technique above indicated, were examined in detail. It was ascertained that narcotization greatly simplifies the study of familiar details—cyclosis, functioning of the vacuoles, structure and activities of the œsophagus, and feeding. Several new observations and corrections were made; these are indicated below with the topics to which they refer.

In a narcotized paramecium, lying with the oral groove up, and the anterior end pointing to "twelve o'clock," there can be seen a steady and vigorous cyclosis extending anteriorly along the right fold of the body (anterior stream) and posteriorly along the left fold (posterior stream). The writer noticed that the cyclosis may

at times accelerate at some part; this acceleration is local and limited and soon traverses the entire course, ceasing first where it began. The acceleration seems independent of the functioning of the contractile vacuoles, since it occurs at intervals much greater than those of vacuole closure. The contractile vacuoles, fixed to the ectosarc, can be seen to operate, as in normal animals, at intervals of from ten to twenty seconds.

The gullet of *Paramecium* is usually represented in the literature as a short and narrow tube tapering posteriorly to the region of the aggregation of food particles, at which position it expands into a sac of somewhat greater diameter than the gullet proper. This description is doubtless referable to the fact that in the dead animal the œsophagus presents an appearance quite different from that in the living. In specimens carefully killed, fixed and stained the gullet was found to be scarcely recognizable, although the other structural details seemed unimpaired. In animals freshly killed, but not otherwise treated, the gullet presents the appearance indicated in the literature. The writer has often observed that in paramecia being killed by dilute poisons a convulsive rearrangement in the protoplasm in the posterior half of the body chokes up the œsophagus and tends to elevate the oral depression. The gullet, therefore, shortens and constricts, and if a new food vacuole is aggregating at this time the distal end of the gullet naturally remains relatively larger than the shrunken duct itself.

In a narcotized living paramecium the gullet can be observed as a smoothly curved and posteriorly tapering tube, approximately one fourth the length of the body. It extends from the peristome posteriorly toward the aboral side, but not through the middle of the body, so that the distal end lies near the ectosarc; and as seen from the oral surface and the posterior end, it twists obliquely to the left of the longitudinal axis of the animal (Fig. 1, *a, b, c*).

The undulating membrane, or ciliated lining of the œsophagus, was superficially studied in a few animals which had stopped swimming, but still rotated feebly, revealing the gullet in all its aspects. In these animals the undulating membrane was still functioning rapidly, though apparently somewhat slower than in a normal paramecium. The undulatory motion can be likened to that of at-

tached ribbons blown in front of an electric fan, or to that of the succession of waves obtained by shaking and twisting a number of fixed ropes. In no case, however, even at the moment of the cessation of undulatory motion, was it possible to observe in the gullet the cilia themselves; only their wave-like motion could be

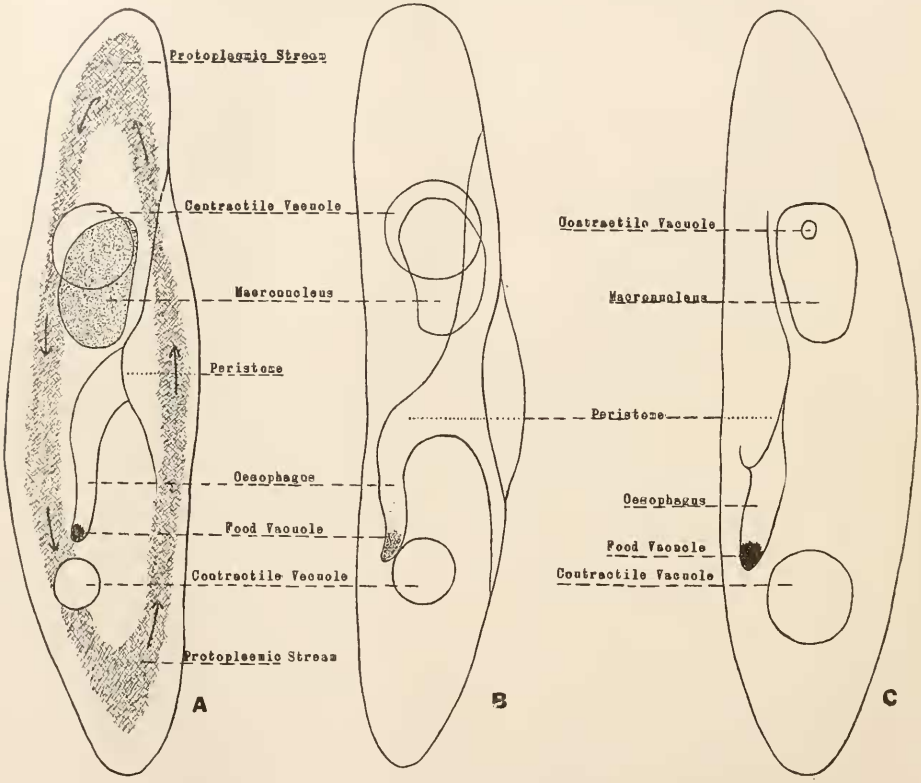

FIG. 1. Camera lucida tracings of specimens of *Paramecium caudatum* narcotized with propyl alcohol, intended to give especially the appearance of the gullet as the animal turns through 180°. The paramecia are heavily narcotized, and the incorporation of food vacuoles is beginning to be retarded.

A. View of paramecium in the position most frequently assumed when quiet. The gullet is in semi-profile. Food particles are aggregated, but remain in the gullet.

B. View of paramecium lying on its "side." The gullet is in full profile. Food particles are not aggregated.

C. View of paramecium from the aboral side—turned through 180° with reference to the position in (*A*). Food particles are aggregated, but remain in the gullet.

seen. The activities of the undulating membrane, in directing the passage of "food" particles (Chinese ink) to the vortex at the distal end of the gullet, and in returning unaccepted particles to the peristome, are rendered easy of observation by the quieting action of propyl alcohol. It may be, however, that the normal process of food acceptance is modified by the drug when sufficient of the latter is employed to effect a complete narcotization.

Summary.

1. Normal and iso propyl alcohols, in concentrations around two and three per cent., respectively, are superior narcotizing agents for *Paramecium*.

2. In narcotized paramecia it is possible to observe with convenience the structures and activities characteristic of the organism.

3. The cyclosis is not a uniform flow, but is subject to waves of acceleration.

4. The œsophagus is a much more extensive structure than is generally represented in the literature.